Design and Development of Passive Lab-On-Chip Plasma Separator Using Fused Filament Fabrication Technique and Analysis of the Parameters Affecting the Extraction Efficiency

Synopsis

submitted in the partial fulfilment for the requirement of

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in

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Submitted by

Shamima Khatoon

Under the supervision of

Dr. Gufran Ahmad

Assistant Professor

Department of Electrical Engineering, Faculty of Engineering

Dayalbagh Educational Institute (Deemed to-be University)

Dayalbagh, Agra (UP) – 282005

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1. INTRODUCTION

1.1. Lab-On-Chip Devices or Micro-Total Analysis System

The Lab-On-Chip (LOC) Devices combines the protocol of analytical chemistry viz. Sample Collection, Sample Preparation, Bioseparation or Bioseparation, Sample Transfer for Analysis, Detection and Results into a single automated, compact, miniaturized measurement device which is also called Micro-Total Analysis System (μ-TAS) [1]. The steps of analytical chemical laboratory or pathology for Target Analyte Detection is shown in Figure 1(a), whereas block diagram and assembly layout of LOC Device is shown in fig. 1(b) and (c) respectively. LOC Detection offers various advantages like low requirements of samples and reagents, including field testing, Point-of-Care Testing (POCT), patient's bed-side testing, rapid operation, high convenience and low cost [2].

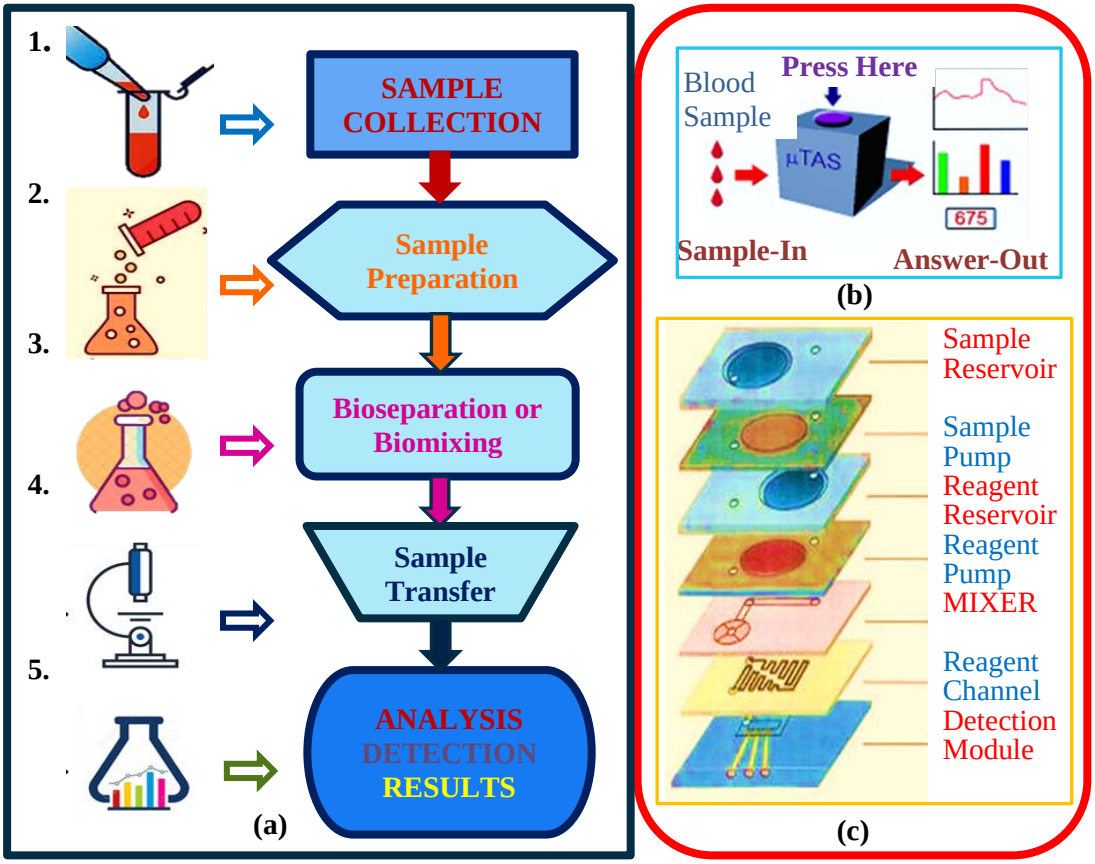

Fig.1 (a) Protocols of Analytical Chemistry; μ-TAS (b) Block Diagram ; (c) Assembly Layout

These LOC Devices can process microliters quantity of blood sample and provide rapid result to the operator. Any kind of LOC Device consists of two subparts viz. Micro fabricated Integrated Channels and chambers and a microfluidic pumping unit for fluidic movement. LOC can be uniquely design for specific type of Detection to perform dedicated function or set of functions [3].

1.2. Mircofluidics and Bioseparation

Microfluidics are the channels ranging from few tens to hundreds of μm that can process or manipulate 2-5ml of fluidics. Microfluidic Chip can contain no. of units such as sample inlet pump, reagent well, mixers, separators, reaction well, transport channels and detection area to perform a dedicated or set of function on the sample under observation. Bioseparation is a very significant process of Microfluidic Devices that affects the later stages of Sample Analysis such as Detection Accuracy and Results of the Target Analyte Detection Cycle [4]. Plasma Extraction is one of the very crucial Bioseparation process for proper functioning of Microfluidic Device. Without Plasma Extraction Microfluidic chip can anytime malfunction to give unexpected output or false results. The Schematic of Microfluidic Device is shown in Figure.2 (a), while actual photograph of market ready Microfluidic chip is shown in Figure.2 (b) [34].

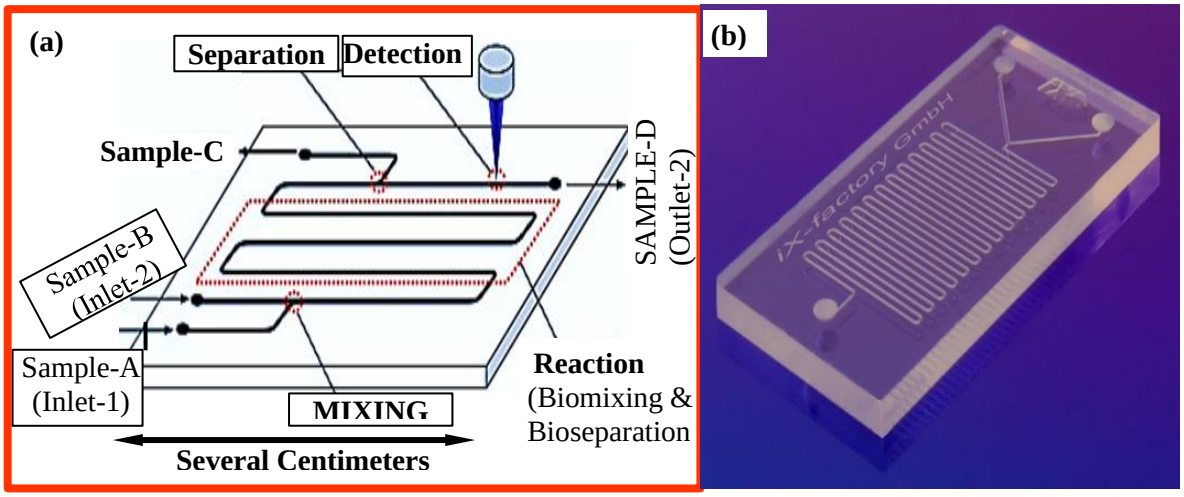

Figure 2: Microfluidic Device (a) Schematic (b) Chip Photograph

1.3. Blood Components and Plasma Separation Techniques

Whole Blood consists of two parts, various cells like Red Blood Cells (RBCs), White Blood Cells (WBCs), Platelets and Plasma. Plasma is the watery parts that constitute 55% of whole blood. The volumetric percentage of Plasma w.r.t. whole blood is shown in Figure. 3(a)). Plasma contains all kind of proteins, enzymes, biomarkers, DNA, RNA, ions, electrolytes, proteins, etc. which provides wide range of information regarding various health conditions and associated medical illnesses [5]. Blood and Plasma components are shown in Figure. 3(b) and Figure. 3(c) respectively. Plasma Protein tests are very common in Laboratory to find out many kind of proteins deficiencies. Hence plasma is an

important clinical sample to determine almost all types of medical conditions, infections and diseases. The block diagram representation of broad classification of Plasma Separation Techniques is shown in Figure. 4.

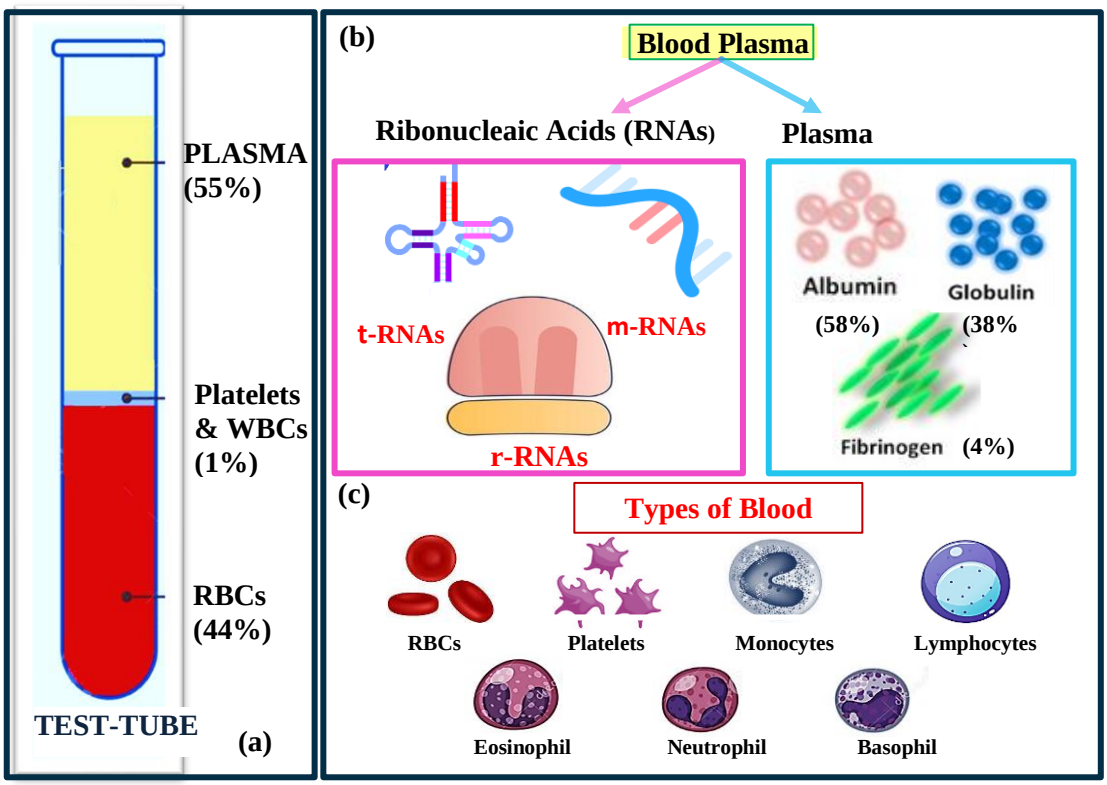

Figure 3 (a) Schematic of RBCs settling at the bottom of Centrifuge Tube, while retaining Plasma above the serum; (b) Schematic Diagram of Plasma Components; (c) Schematic Diagram of Blood Components

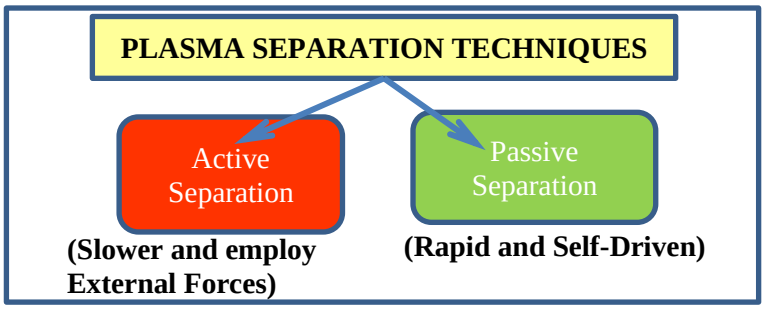

Figure 4: Block Diagram of Broad Classification of Plasma Separation Techniques

Active Separation employs external forces like acoustics [6], dielectrophoretic [7], magnetic [8], centrifugal force [9] or combination of above forces, while Passive Separation is based on cell properties like cell shape, size, stiffness, weight etc. to guide target cells towards the dedicated direction or position. Sedimentation of heavier cells like RBCs, WBCs and Platelets generally leave Plasma (watery part and lighter) at the top of test tube as shown in fig. 3 (a). Hence sedimentation strategy combined with either

active or passive techniques can extract pure plasma with higher extraction rate. Examples of Laboratory accepted standard methods of Active Plasma Separation Technique employs “Centrifugal Assisted Sedimentation” that are either “Centrifuges-based (Vortex Mixer-based)” or “Compact-Disc (CD) - based”.

Passive Cell Sorting Techniques based on flow cytometry are biomimetic Separation [10], gravitation-assisted [11], Deterministic lateral displacement [12], sedimentation [13], pinched-flow fractionation [14] etc. On the contrary microstructure based cell sorting methods are Membrane Separation Techniques (pores and nano-fibres) [15], Dead-end Filtration [16], Cross-flow Filtration [17], Tangential Flow Filtration [18], RBC sorting using immunocapture [19], Retarded Flow and Wetting Contrast [20], Micro-obstructions like comb-type[21] and mesh-type [22] or a combination of any of these techniques to filter out unwanted heavier blood cells such as erythrocytes and leukocytes.

Based on the mode of operation the passive separators are further divide as-

(i) Microfluidics-based (Figure. 5) that employed hydrodynamics principle of fluid flow, for example plasma follows bifurcations through Cell-Free Region due to Newtonian Behaviour, differential wettability of blood and relative velocity and viscosity of Plasma w.r.t. viscoelastic blood cells [23].

(ii) Microstructure-based (Figure. 6) that employs size selection trapping and filtration through microstructures (like fins, comb-type, micropillar-arrays etc) and Microporous Membrane-based [24] (Vivid™ Plasma Membrane, Glass Fibre Membrane, Polymer membrane, etc.)

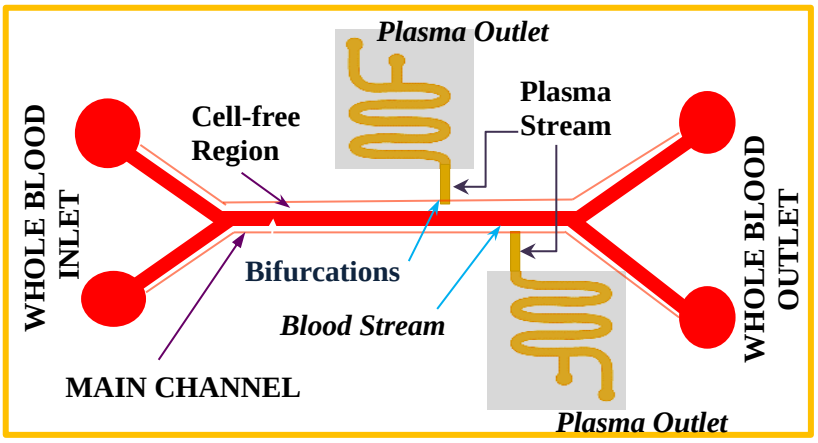

Figure 5: Schematic of RBCs settling at the bottom of Centrifuge Tube, while retaining Plasma at the Top

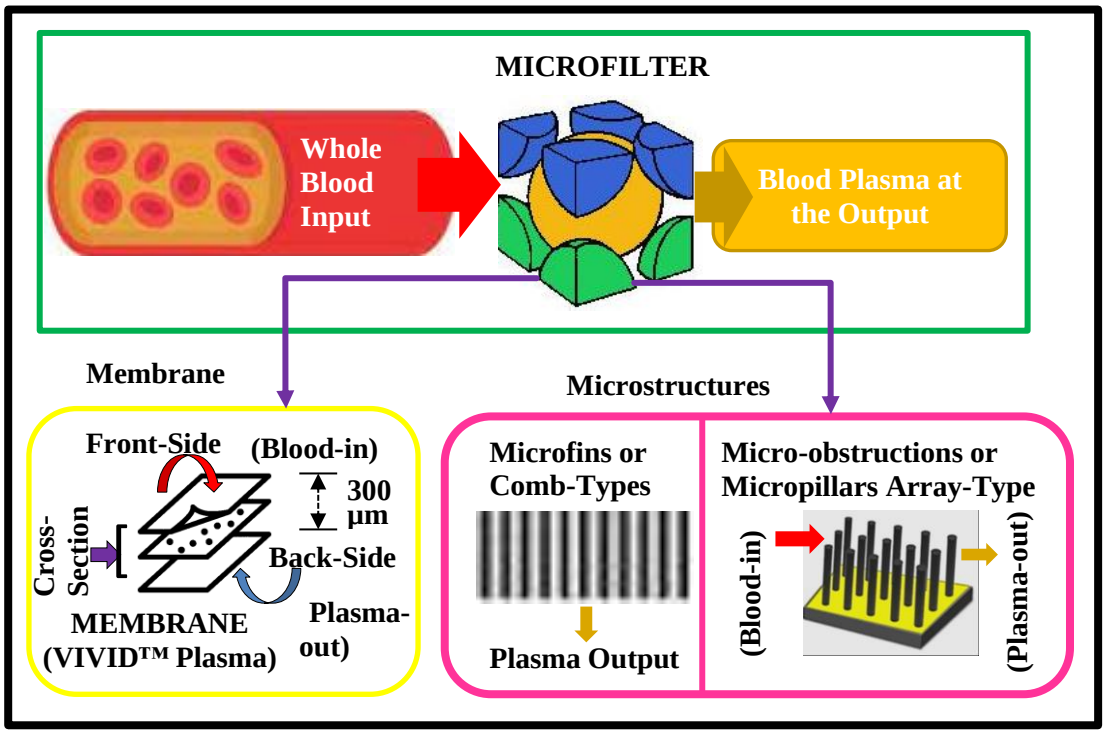

Figure 6: Schematic Diagram of the Plasma Separation through Passive Techniques employing Membrane and Microstructures

1.4. Centrifugation Based Plasma Separation

The Centrifuges and Compact-Discs are basically universally accepted laboratory methods of Plasma Separation based on Active Techniques. These are electromechanical devices in which whole blood sample is either kept inside a test-tubes or sedimentation chamber of CD that are rotated at high starting velocity generally 3800 rpm to generated a Centrifugal Force high enough to assist the sedimentation of the heavier blood cells like RBCS, WBCS and Platelets [9]. The sedimentation rate depends on the various parameters of the cells inside the sample and the structure of the rotating device.

As described by Stokes' Law, the sedimentation rate of a particle, vr, due to centrifugal force is:

$$v_r = \frac{dr}{dt} = \frac{\left(\rho_p - \rho_m(r)\right).d^2 \,.\, \mathrm{r}.\omega^2}{18.\eta_m\,(r)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad \text{(I)}$$

$$\text{rpm} = \sqrt{\frac{RCF}{2.839\times10^{-5}\,.r}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad \text{(II)}$$

Where $\boldsymbol{\rho_p}$ is the particle density, $\boldsymbol{\rho_m}$ **(r)** is the media density at a given r, **d** is the diameter of the particle, **r** is the radial distance from the rotation centre, **ω** is the angular velocity, $\boldsymbol{\eta_m}$**(r)** is the media viscosity at a given radius and **RCF** is relative centrifugal force.

These devices can extract pure plasma without any residual cell within 5 minutes of operation but requires continuous electrical supply and bulky mechanical platform along with precise control of rotational velocity to retain plasma purity and cannot be employed for patient's bed-side POCT. Centrifugation assisted Sedimentation based Active Plasma Separation mechanism is elaborated in detail in Figure. 7.

The advancement from CD-based microfluidics towards slanted-spiral microchannel and multiplexed slanted-spirals [24] assists ultra-fast rapid extraction, improving the flow rates from 1.5 ml/min to 24 ml/min.

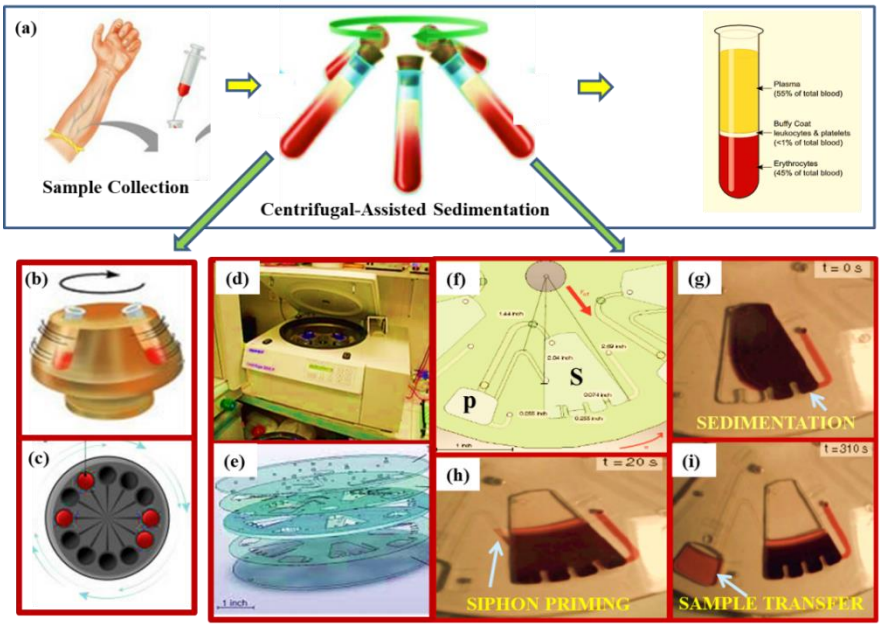

Figure 7: Centrifugal-Assisted Sedimentation based Active Plasma Separation; (a) General Description of the Mechanism [9]; (b) Schematic Diagram of the Plasma Separation using Centrifuges; (c) Schematic Diagram of the Top-view of Centrifuge; (d) Compact-Disc Based Laboratory Device for Plasma Seapartion; (e) Multilayer CD-Based Plasma Separation Device; (f) Schematic Diagram of CD-Based Device depicting Sedimentation chamber (S) and Plasma Chamber (P) connected via Microfluidic Channel; (g) Whole Blood inside the Sedimentation Chamber; (h) Siphon Priming with Blood Plasma after subjecting to high rotational velocity; (i) Sample Transfer after Plasma Extraction at the Plasma Chamber.

2. MOTIVATION

2.1. Need of Plasma Separation-

(i) Plasma Specimens can be processed more quickly, shortening the turnaround time for POCT, reflecting accurate in-vivo state of the patients. This can help doctors to make quick decision for on adaptable drug dosage especially for critically ill patients to improve their survival rates.

(ii) Plasma Contains Protein “Antithrombin (AT-III)” which a natural anticoagulant makes it perfect for Microfluidic Application. The anticoagulation property of the Plasma makes it a better free-flow fluid, with higher wettability contrast and lower density as compared to whole blood or serum.

(iii) Plasma separation from whole blood at a very first step retains the sample quality producing detection results with higher accuracy. This is because there is no need to externally add artificial anticoagulant like heparin or EDTA, which deteriorate the sample quality and hampers the detection results.

(iv) Plasma Samples because of the reasons explained above are more compatible and perfect for Lab-On-Chip Microfluidic Devices as compared to whole blood or serum.

(v) On-Chip Plasma Testing can improve the speed and limit of detection especially when the target species are the Viruses.

(vi) Plasma Testing forms the bases of Biosensing Cards with Greater Reliability and reduce chances of Chip Malfunctioning.

(vii) Plasma Samples are much easier in handling and storage as compared to whole blood sample

2.2. Significance of Qualitative Rapid Antigen Testing

The schematic of disease control cycle through Rapid Antigen Testing is shown in figure.8 and its key benefit are listed below-

(i) Compatible to Resource limited rural areas.

(ii) Preferred for Rampant disease management.

(iii) Mass detection and disease control in Pandemic Situations like COVID-19.

(iv) Early detection and control at the very first acute stage of infection.

(v) POCT and faster health recovery.

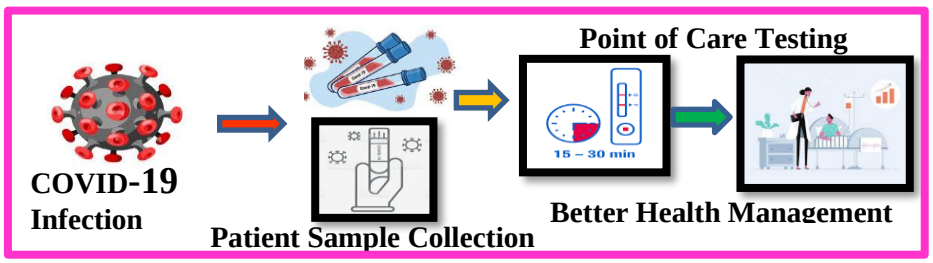

Figure 8: Schematic showing faster health management and disease control through Rapid Antigen POCT.

3. SURVEY ON PLASMA SEPARATION TECHNIQUES AND RAPID ANTIGEN POCT

3.1. Literature Survey on Plasma Separation Techniques

The literature survey shows remarkable developments in the area of Active and Passive Techniques of Plasma Separation and it has been observed from the study of related researches that most of the experimental researches were done on the device structure and fabrication technology. The Active Plasma Separators fabricated with Microfluidics and Passive Separators fabricated with LOC Architecture are reported in this literature survey. The on-chip plasma separators based on passive methods prove to overpower the laboratory based or other Active methods owing to numerous advantages such as compatibility to biosensing platform, rapid extraction, compact lightweight and self-powered design makes them suitable for POCT and resource limited environment. The key parameters on which the researchers are focusing for improving the on-chip plasma separators are basically plasma purity, plasma extraction efficiency, Microfluidic channel hydrophobicity, Contact angle between fluid and channel surface, different hydrodynamics principals (dead end, lateral flow, cross flow, RBCs retarded flow, wettability contrast of plasma, constriction effect, etc.) and separation science (RBCs immunocapture, size-selection trapping, symmetrical filtration, etc.)

3.1.1. Microfluidics CD-Based Sedimentation-Assisted Active Plasma Separators

The working principle of all CD-Based sedimentation assisted separators is based on precise control of spin profile as shown in fig.6 to extract plasma free from residue cells.

(i) Amasia and Madau et.al in 2010, fabricated a multilayer compact-disc centrifugal microfluidic device for Plasma Extraction from 2ml of Whole Blood sample in 2.5 min. This prototype device was fabricated using traditional plastics machining techniques, with an extension to low-cost manufacturing techniques (i.e., injection molding) [9].

(ii) Moore et al. in 2010, fabricated capillary valves in the centrifugal microfluidic disc by designing patterned surfaces, emphasizing that the 3D Printing Techniques can be used as an alternative to Photolithography and CNC machining [25].

3.1.2. Passive Lab-On-Chip Plasma Separators fabricated using PDMS Soft Lithography

(i) Sara et.al [26] (2006)- Separation Membrane-assisted Microfiltration and capillary force driven plasma extraction of high purity in lesser time (Figure. 10)

(ii) Park et.al [27] (2015) – Retarded flow-assisted sedimentation, microfiltration and free-flow wetting of ethanol-treated collection channel (Figure. 11)

(iii) Madadi et.al [28] (2015) - Separation Science, Fluid Dynamics, and Blood Rheology (Figure. 12)

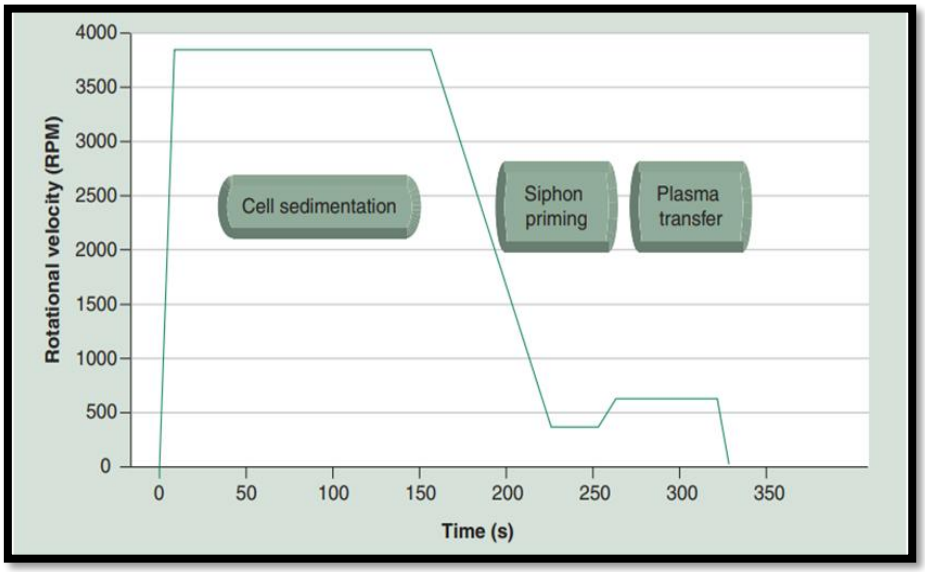

Figure 9: Spin profile graph detailing rotational velocity over time during the on-disc plasma separation process [9].

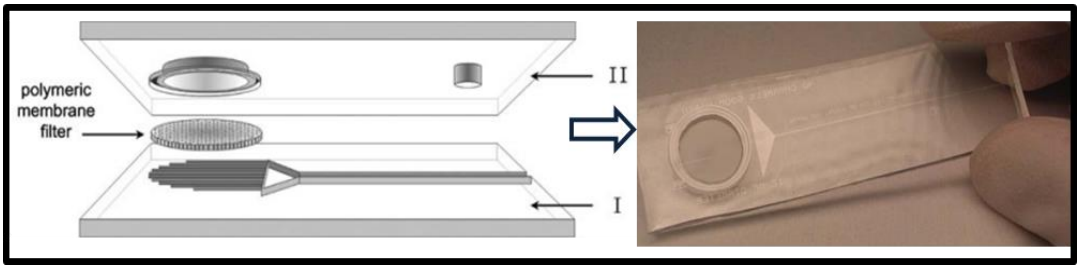

Figure 10: Membrane-based Passive Blood Plasma Separator Device; (a) 3D Schematic; (b) An Assembled PDMS/Glass Plasma Separator [26]

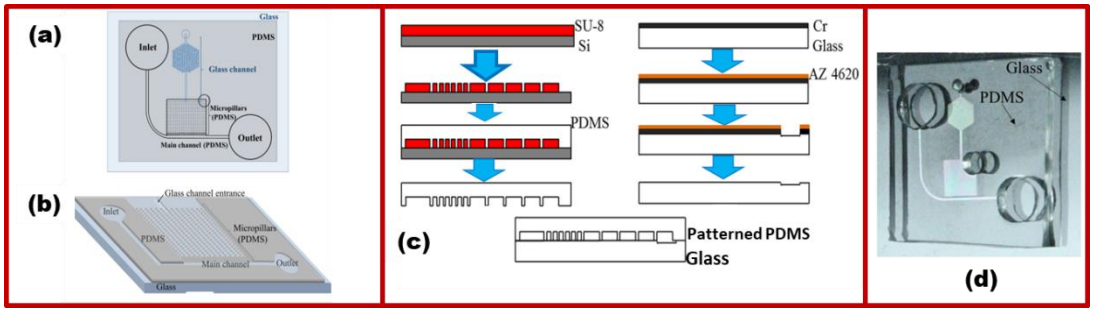

Figure 11: Passive Blood Plasma Separator Device; (a) Top View; (b) 3D Schematic; (c) Fabrication Steps; (d) An Assembled PDMS/Glass Plasma Separator [27]

3.1.3. Devices Fabricated using Hybrid Techniques

Sollier et.al [29] (2009) - Innovative device is based on the lateral migration of red cells and the resulting cell-free layer, which is used to supply geometric singularities (an ear-cavity or a corner-edge) and

locally enhanced clear plasma region (Figure. 13). The device fabrication is based on the integration of hybrid chips using Standard Photolithography and PDMS Soft-Lithography.

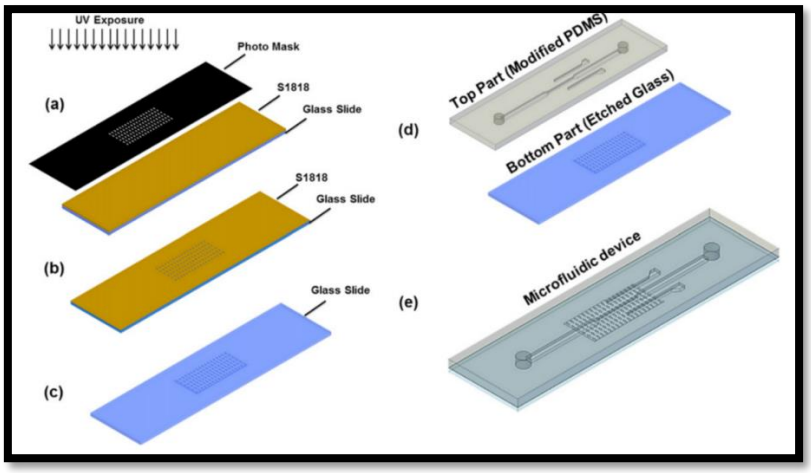

Figure 12(a) MIMPs Filtration Channel Manufacturing Steps (b) Micro Device's top and (c) bottom part (d) before and (e) after plasma bonding [28]

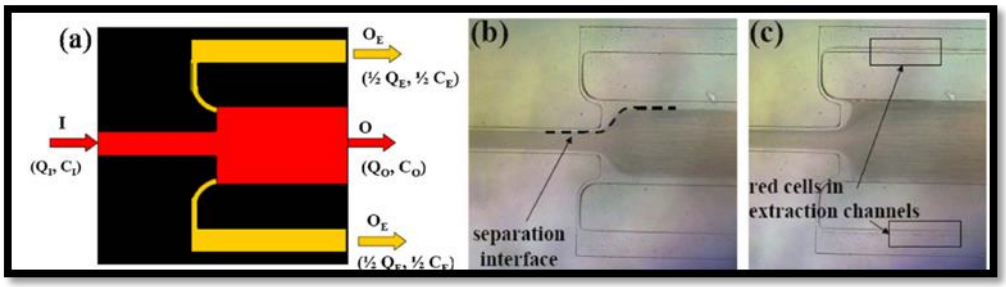

Figure 13: Superhydrophobic Clamshell Plasma Separator; (a) Blood Introduction (b) Cell Gravity Sedimentation (c) Plasma Collection (d) Micropillar Array at the depression of the top cover [29]

(i) Maria et.al [30] **(2016) -** Capillary flow of blood in a microchannel with differential wetting, to induce a self- built-in filter for plasma separation and subsequent chromatographic detection of glucose (Figure. 14). The device fabrication is based on the DLP 3D Printing, SU-8 Photolithography followed with PDMS Soft Lithography.

3.2.3. CNC Micromachining

Su et. al [19] **(2020),** modular universal plasma-separation microdevice based on immunocapture and size filtering as shown in Figure. 15(a).

3.1.4. DLP 3D Printing

Liu et. al [31] **(2015)** introduces membrane based superhydrophobic clamshell style Plasma Separator. Gravitation assisted sedimentation and membrane-based filtration, while Superhydrophobic Surface-assisted Size extraction of plasma Figure. 15(b).

3.1.2. SLA 3D Printing

Kim et. al [32] (2022) – Glass fibre filter based erythrocyte binding, self-pressure driven plasma separation and multiple LFA diagnosis as shown in Figure. 15(c).

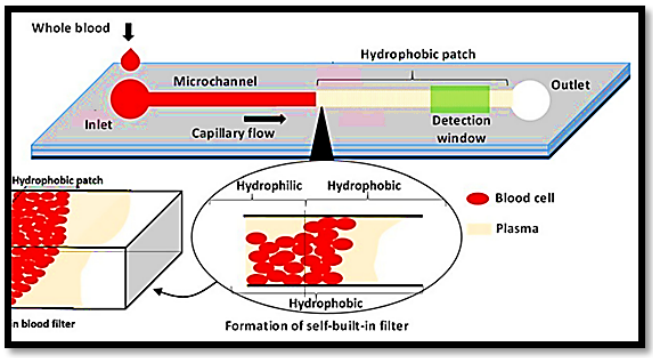

Figure 14: Superhydrophobic Clamshell Plasma Separator; (a) Blood Introduction (b) Cell Gravity Sedimentation (c) Plasma Collection (d) Micropillar Array at the depression of the top cover [30]

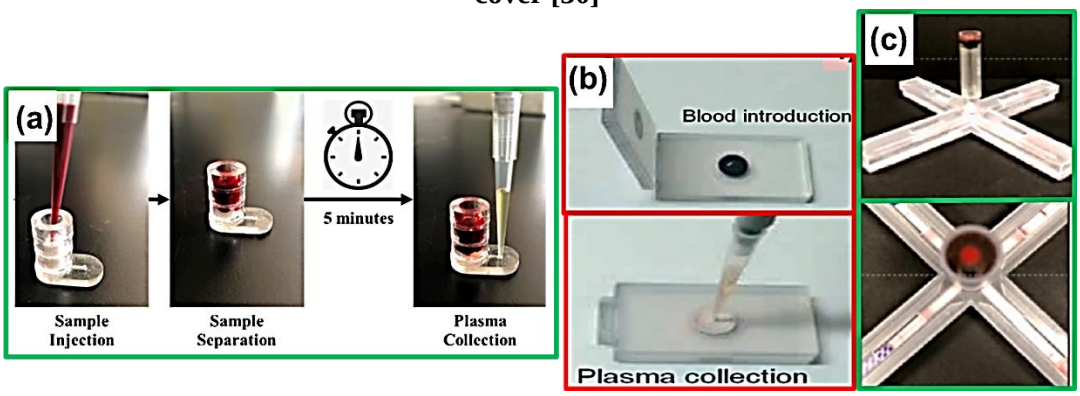

Figure 15 (a) Plasma Separator based on Immunocapture [19] ; (b)Superhydrophobic Clamshell Plasma Separator [31]; (c) Pressure driven Separator with Multiple LFA strips [32]

3.2 Ground Survey on Rapid Antigen Biosensing Card Testing

Images of some commercially available Rapid Biosensing Cards are shown in Figure. 16

(i) **Place Visited-** U.P. East Rural Areas (Atarra, Naraini, Harha, Kaboli, Kalhara, etc at Dist. Banda)

(ii) **Test Sample Type/Separation Technique**– Serum /ACD Plasma (Electromechanical and Chemical Separation Technique) employing Centrifuge Tube based Active Separation.

(iii) **Target Diseases and Other Routine Test-** Covid-19 (only at Govt. Hospital), Dengue, Malaria, HBV, HCV and HIV-AIDs. Routines tests like β-HCG, HbA1c, TSH, LFT by POCT Analyser

(iv) COVID-19 TESTS- At CHC by IgM/IgG Combo kit.

(v) Rampant Disease – MP

(vi) Target Diseases diagnosed via card methods only- Dengue and HIV

Table 1. Comparison of the Device Structure, Separation Technology and Extraction Efficiency of some of the Passive Lab-On-Chip Plasma Separators Devices

Plasma Separator	Device Structure	Separation Technology	Efficiency/ Analyte
Sara et.al [26] (2006)	Two layered PDMS Device with Membrane filter (Polypropelene) incorporated between the lid and the bottom substrate. The bottom substrate contains forty microchannels to collect the filtrate to guide it towards the collection channel.	Propose a filter material that minimizes nonspecific analyte adsorption, both in the free and protein-bound form.	20 µL whole blood intake.PBS-buffered testosterone solution and testosterone spiked human plasma were filtrated and analyzed with RIA
Park et.al [27] (2015)	Patterning of PDMS to form Micropillar array employing Soft-Lithography on the UV developed and Etched SU-8 Mold for retarded flow and microfiltration. Patterning of Glass via etching to developing microchannels for Plasma Collection.	Retarded flow assisted sedimentation and filtration of RBCs and WBCs, through Array, while free flow wetting of Plasma through the ethanol treated microchannel.	16nl of pure Plasma out of 15µl of whole blood. Experimental Model solution filtering out 4.5µm of PS Beads.
Madadi et.al [28] (2015)	The clogging delay caused by RBCs in a hydrophilic PDMS channel and the symmetric out-of-plane cross-flow filtration MIMPs, exploited to maximize the extracted plasma from undiluted blood.	Separation science, fluid dynamics, and blood rheology. Shear force act on the main channel while the capillary forces exerted on the plasma collection channel.	Extracted 0.1µl of plasma from 5µl of blood TSH qualitative testing employing diagnostic kit.
Maria et.al[30] 2016	DLP 3D Printing, SU-8 Photolithography followed with PDMS Soft Lithography. The main PDMS microchannel designed to be hydrophilic near the inlet side, and hydrophobic at near the detection window.	Capillary driven PDMS channel with dual wettability nature act as a self-filter for plasma extraction exploiting differences in the viscosity and dynamic fluid velocities of the Blood and Plasma.	450nl pf plasma was extracted. Plasma recovery efficiency was 22.5%. On-Chip Detection of Glucose.
Su et.al [19] (2020)	Cup-shaped primary separation chamber (outer) loaded with Anti-RBC soaked acetate fiber pillar matrix (inner) and holds the blood sample. The final purification (bottom) chamber holds the Separation Membrane (VIVID™ GX), connects the primary chamber and the plasma collection outlet.	Acetate fiber matrix allow RBCs Immunocapture. GX- Membrane allow size selection trapping of WBCs and Platelets	Extracted 100 µl of Hemolysis-free 100% Pure Plasma from 400µl of whole blood sample. Quantitative PCR HBV Testing. Glucose recovery rate is 100±0.73%
Kim et.al [32] (2022)	The separation device consists of a barrel and a plunger. Barrel holds the diluted whole blood sample. Plunger holds the glass fiber filter. Multiple LFA strip holder/house cover provided to hold rapid diagnostic kits	Set of seals, self- pressurize the flow through the pored-matrix, binding the erythrocytes on the filter surface readily extracting Plasma.	Multiple assay diagnostics HIV Ab HBVs Ag HBVs Ab HCV Ag

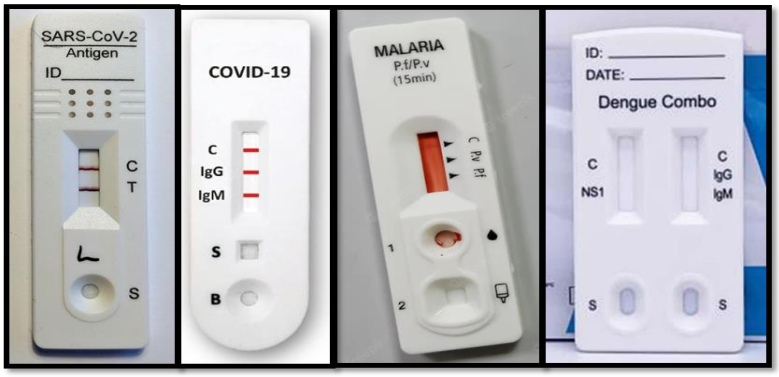

Figure 16: Commercially available Rapid Antigen Testing Kits (not recommended for self-testing)

3.2.1. Cumulative Record of the Target Disease

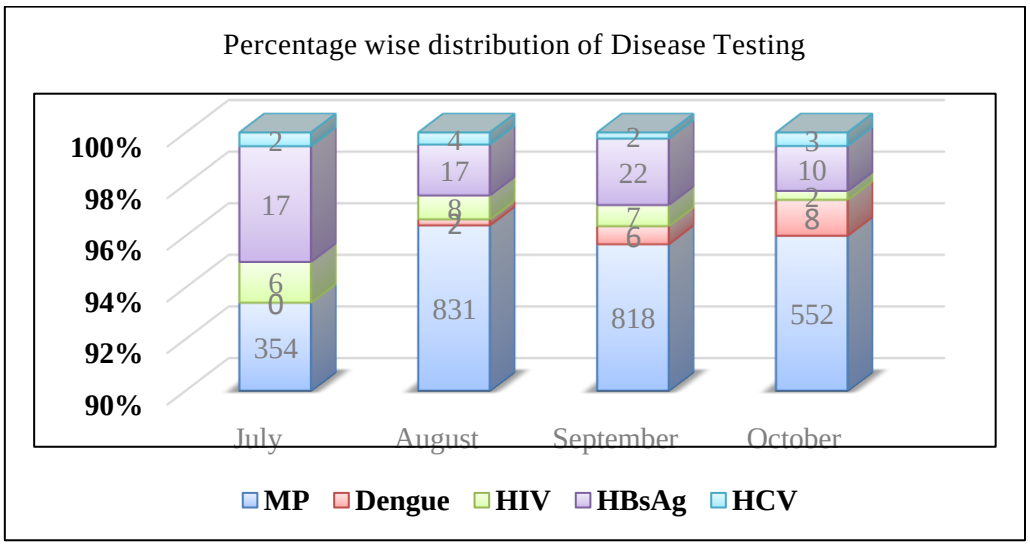

Figure 17: The month wise- cumulative record of Laboratory Diagnosis of Target Diseases

3.2.2. Card-Testing Statistics at the Place of Visit

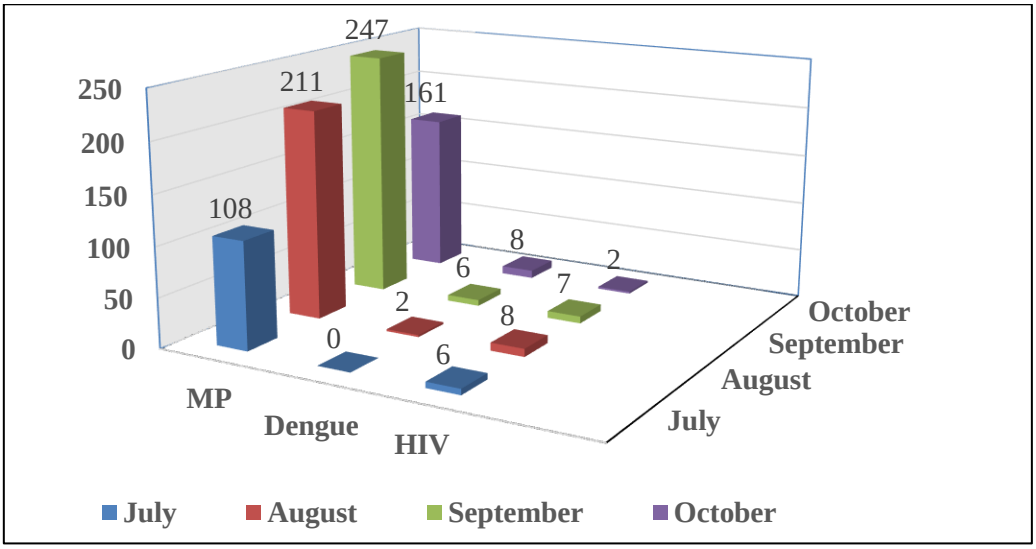

Figure 18: Month-wise breakthrough of Diagnosis based on Card Methods

4. GENERAL DESCRIPTION OF THE PROBLEMS IDENTIFIED

4.2. Problems Identified in Sample Preparation for Diagnosis of Diseases

(i) Plasma Separation needs repeated **3-4 cycles** of mechanical, electrical and chemical treatment along with **large volume of the whole blood sample.**

(ii) **Chemical Treatments** affects the viability of the diagnostic results because the analyte (Target Species) concentration is degraded results in **Lower Limit of Detection** and viral concentration.

(iii) **Mechanical Treatments** are time consuming and require bulky equipment which are not suitable for POCT.

(iv) **Electrical Treatments** needs dedicated power supply and mechanism to control the extraction not suitable for rural areas.

(v) Requirement of Equipment-free new techniques for bioseparation with minimum or no external treatments.

4.3. Problems Identified in the existing on-chip Plasma Separation and Detection Techniques

(i) Extremely Slow turnaround time for Rampant Diseases results in poor disease control.

(ii) On-chip plasma separators are mostly researched for the detection of hormones like TSH, β-HcG and Glucose level.

(iii) On-Chip Plasma Separators extracts only microliters plasma, which is not suitable true detection of Target Pathogens.

(iv) Fabrication techniques which are economical, easier and faster must be explored for commercialization of these Devices.

(v) High Plasma yield and purity can be achieved by integrating Separation Membranes and pump-free Microfluidics LOC Devices

5. RESEARCH GAPS FOUND

5.1. Research Gap found in the Fabrication Technology

(i) The Material Extrusion in Filament form that is the simplest and economical genre of AM Technique is proposed to be utilized for the fabrication of device.

(ii) The FDM 3D-Printing technique is still not utilize for this biomedical application because of various challenges like poor strength, surface roughness, uncontrolled wettability and dimensional

errors, which are the key problems to be addressed in terms of Device fabrication and optimization.

5.2. Research Gap found in the Device Structure

(i) The device structure to be proposed so that the drawbacks of the FDM 3D Printing technique do not affect the device performance.

(ii) The key mechanical characteristics of a biomedical device to be incorporated.

(iii) Avoiding the conventional post-processing techniques to retain the structural details while at the same time improving the appearance and strength.

5.3. Research Gap Found in the Separation Science-

(i) Poor extraction efficiency with large starting volume of whole blood, so the factors that will improved the plasma volume with low starting volume of blood must be explored.

(ii) The Microfluidics approaches are usually slower and needs structure with which precise control in surface qualities and external pumping mechanism for higher purity plasma extraction.

(iii) The Membrane based approaches are generally faster, pump-free but prone to haemolysis.

(iv) (iv)Therefore, a hybrid approach integrating benefits like extraction efficiency, higher plasma purity of Microfluidics-based Separation and faster pump-free approaches of membrane technology are the area of research for this proposal.

5.4. Research Gap Found in the choice of Target Analytes

(i) On-chip plasma separators are mostly designed for the detection of plasma proteins, hormones like TSH, Beta-Hcg, animal DNA sequence and Glucose level.

(ii) Atleast more than 10 copies of Target Antigens must be needed for viable detection so plasma separator must produce atleast 200 µl of blood plasma with sufficient volume of whole blood keeping in mind an optimum efficiency of 20% approx.

(iii) Multiple LFA strips embedded in a Detection Window for Label-Free and Early Detection of Target Analytes- Ag (COVID-19 and Dengue), MP and routine tests including hormones (TSH,β-HcG).

6. PROPOSED OBJECTIVES OF THE RESEARCH

OBJ 1: To design and simulate the Plasma Separator Device Model on the Multiphysics Platform on the basis of basic fluid flow studies.

OBJ 2: To simulate and optimize the planar parts (3D CAD Modules) of Self-Driven Passive Plasma Separator and fabricate them using FFF 3D Printing Technique.

OBJ 3: To simulate, optimize and fabricate the geometric microstructures suitable to be integrated for bioseparation.

OBJ 4: To fabricate the Integrated Passive Lab-on-Chip Device by assembling the Modules together, to develop the prototype model.

OBJ 5: To simplify the Detection mechanism by Integrating the Proposed Device with the existing biosensing methods (LFAs) and fabricating a Lab-on-Chip Device for *On-Chip Plasma Separation and Detection of Target Analytes.*

7. PROPOSED METHODOLOGY

The methodology to implement the Proposed Objectives is subdivided into three parts-

(i) Fabrication of the Lab-On-Chip Passive Plasma Separator Prototype Device,

(ii) Validation of the device for Plasma Extraction Efficiency on the basis of other key factors affecting it, and

(iii) Testing the reliability of the Plasma Sample to access its suitability for Viral Infection Testing.

In general the fabrication follows standard bottom-up design approach for the basic 3D CAD modules such as Planar Parts and Microstructures. After successful design and simulation of the structure for passive LOC Device, all the modules are supposed to be fabricated using Fused Filament Fabrication (FFF) Additive Manufacturing (AM) Technique. Each Module is mechanically tested and characterized individually on the basis of set of desired parameters, and after successfully achieving them all, these parts can be assembled together to form prototype device.

The flow chart of the Proposed Methodology in brief is given is figure.19

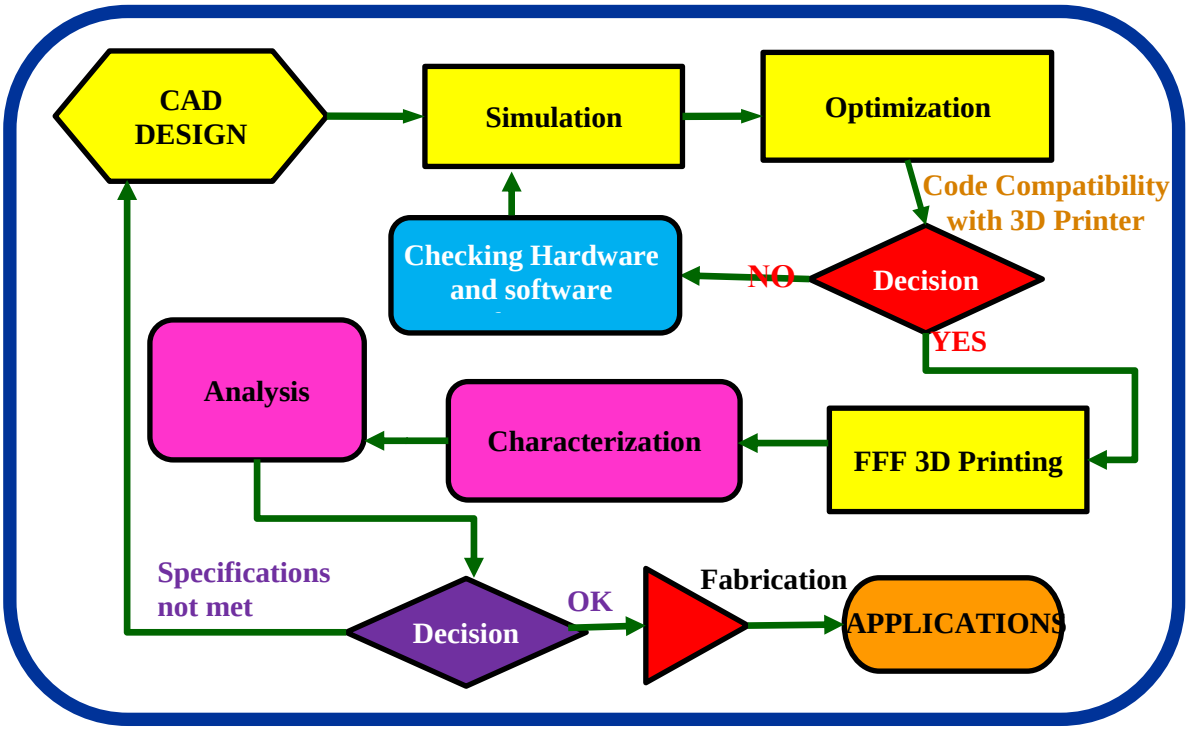

Figure 19: Flow Chart of the Proposed Methodology

8. TIMELINE FOR THE RESEARCH

Table 2: Timeline of the Proposed Research Objectives

TIMELINE OF THE OBJECTIVES	**YEAR 1**		**YEAR 2**		**YEAR 3**	
PROPOSED RESEARCH OBJECTIVES	**Q1 +Q2**	**Q3 +Q4**	**Q1 +Q2**	**Q3 +Q4**	**Q1 +Q2**	**Q3 +Q4**
OBJ 1: Design and simulation on the basis of basic fluid flow studies.						
OBJ 2: Simulation and optimization of the planar parts (3D CAD Modules) using FFF 3D Printing Technique.						
OBJ 3: Simulation, optimization and fabrication of the geometric microstructures suitable to be integrated for bioseparation.						
OBJ4: Fabrication, Integration and assembling of the Passive Lab-on-Chip Device Modules to develop the prototype model.						
OBJ 5: To simplify the Detection mechanism by Integrating the Proposed Device with the existing biosensing methods and fabricating a Lab-on-Chip Device for *On-Chip Plasma Separation and Detection.*						
Thesis Writing, Evaluation and Review and Patent Filing/ Conferences/ Proceedings/ Journals						

9. Lab Facilities At Dei To Carry Out The Research Work (Shown in Figure. 20 and 21)

(i) 3D Printing Rapid Prototyping LAB

(ii) PDMS Soft Lithography Lab Facilities

(iii) Polymer Membrane Casting Assembly

(iv) FESEM Lab Facilities for Characterization

(v) Chemical Laboratory for post processing

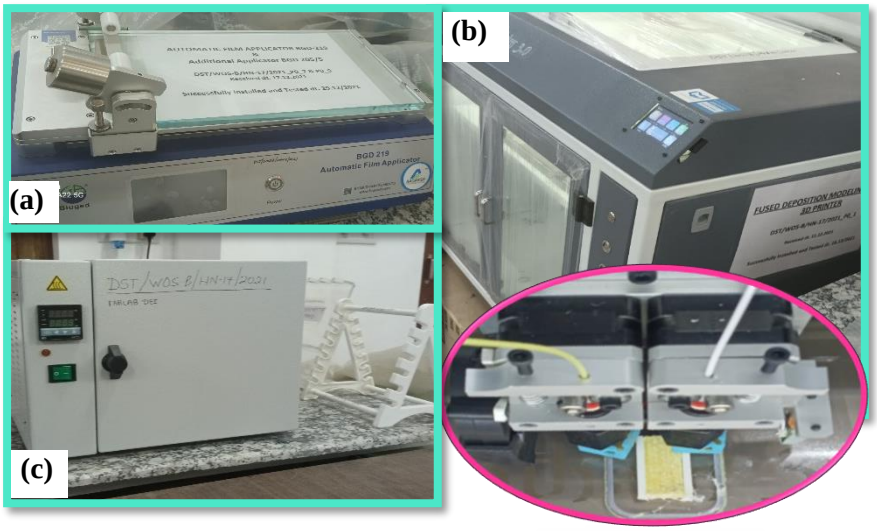

Figure 20 (a) Polymer Membrane Casting Assembly (b) FDM 3D Printer and exploded view of Dual Extruder Sacrificial Printing (c) PDMS Curing Oven

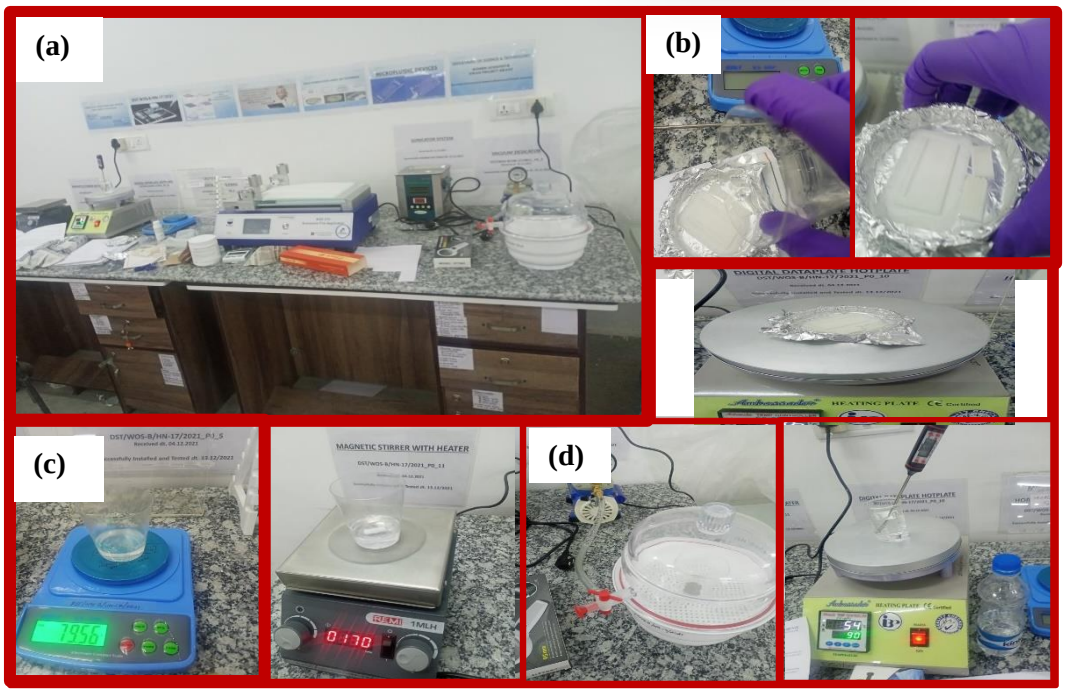

Figure 21 (a) PDMS Soft-Lithography and Chemical-kit Station (b) PDMS Mixture and Molds (c) Analytical Balance and Magnetic Stirrer (d) Vacuum Desiccator and Digital Hot-Plate.

10. WORKDONE SO FAR

10.1. Simulation and Modeling of Microporous Flat Polysulfone Polymer Plasma Separation Membrane (VIVID ™- GX).

Three samples of Vivid™ Plasma Membrane viz front, back and cross-section were subsequently observed in a JEOL JSM-7610F Plus Field Emission Scanning Electron Microscope (FESEM) Apparatus at an accelerating voltages of 1 to 2kV, under magnifications of 100µm for the front-side and 10µm for the back-side of the membrane to observe the randomly distributed pores of asymmetric membrane structure. The observations of pore sizes in µm are shown in Table 2 whereas the workflow is shown in figure. 22.

Table.3 Characterization of Pore Structure for front and back of the samples

Pore Size (µm)	Samples Under Study	
	Front	Back
Min	7.639	0.247
Max	117.708	13.569
Mean (max)	143.225	17.595

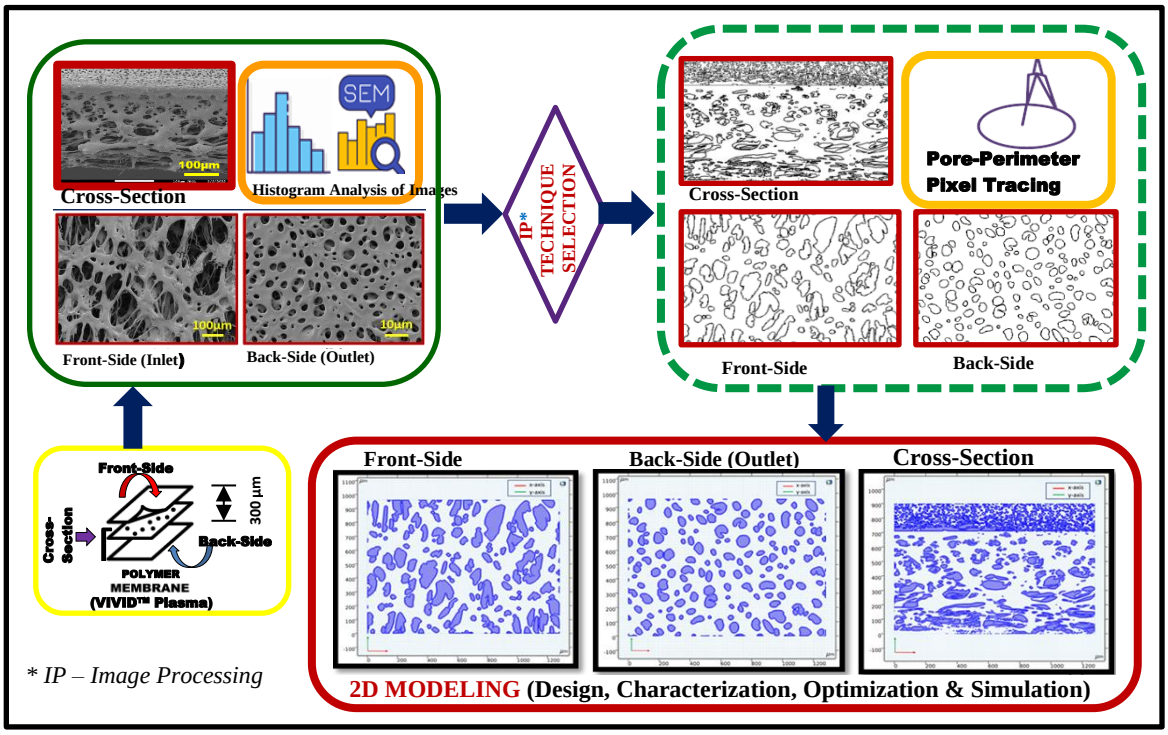

Figure 22 Work Flow of the 2D Modeling and Characterization of Vivid™ Plasma Flat Polymer Membrane.

10.2. Validation of fabrication technology by FFF 3D Printing of Plasma Separator Parts

Premilinary work done on FFF 3D Printed Surfaces and Structures shown in figure. 23.

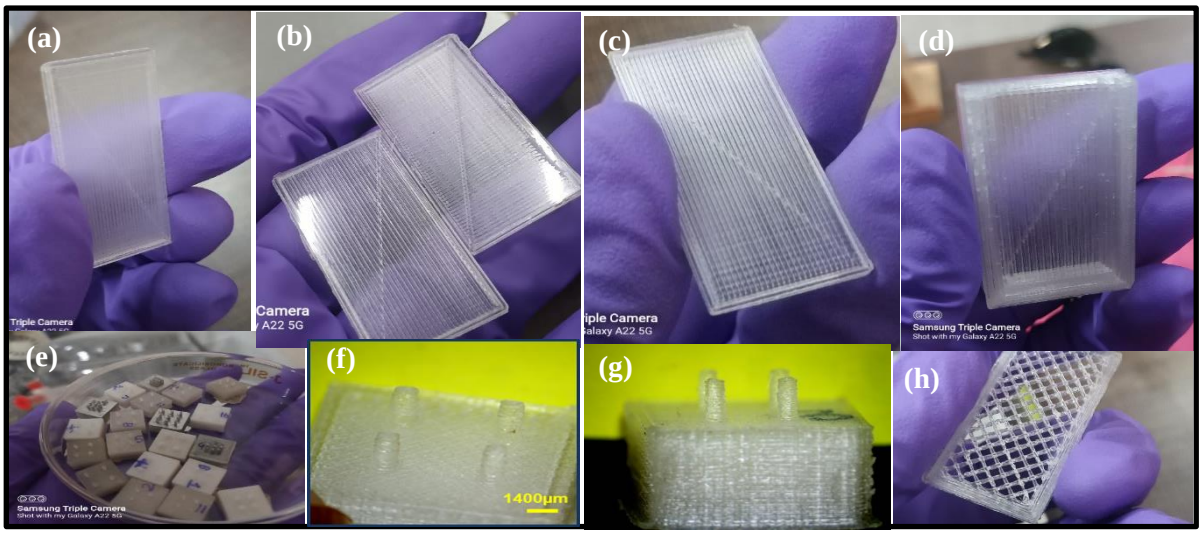

Figure 23 Calibration and Preliminary fabrication work for FFF/FDM 3D Printing showing ; (a) Transparent Surfaces, (b) Highly smooth surfaces; (c) Microchannels (width 23µm) (d) Sample Holder; (e) Samples Parts (39 Nos) with Micropillars; (f) Microscopic Images of Micropillars (800µm dia and 2000µm height), orthogonal view; (g) Side View of Microscopic Image of Micropillars; (h) Supporting Structures.

10.3. Experimental Analysis of Surface Qualities of FFF 3D Printed Surfaces

The optimization at the CAD and machine environment of the FFF AM techniques with can be possible future developments towards the faster and more economical fabrication of the disposable devices and their economic extension. To further support our vision, we fabricated some parts of PMMA-Transparent, PLA-Transparent and Ultimaker PLA+ White. We reported the minimum average roughness of 1.466µm and contact angle 76.5°. The factorial analysis results for two factor ANOVA without replication gives variance σ^2=0.175, and FESEM analysis of surfaces also depicts optimum surface quality.

10.4. Experimental Analysis of Dimensional Errors

3D Printing of Micropillars is performed and two set of samples were prepared. One set of samples (SET-A) is designed keeping pillar diameter of 600µm while other set (SET-B) is designed with 800µm, while keeping height for both the SETS as 2000µm only. We have employed PMMA material extrusion in filament form. Nine samples of SET-A with an array size of four; and two samples of 600µm SET-B with an array size of nine were printed successfully. Optical microscopy was done to measure the diameter along the pillar height at three consecutive points for each pillar. The mathematical analysis of variance for micropillars for SET-A shows a standard deviation of 54.37 µm, standard error of 9.062% and a relative error of 9.09% choosing 0.25 mm nozzle. The study demonstrates that these errors

percentage is still not significant and can be compensated with reverse engineering and effective techniques to neutralize them are proposed in the research such that the device operation will be affected significantly to reduce the extraction efficiency below the proposed percentage.

11. SYNOPSIS SUMMARY

The proposed devices must be fabricated employing FFF AM Techniques with following expected features-

(i) Low Cost Design and Manufacturing.

(ii) High Extraction Efficiency of the fabricated device.

(iii) Compact Rapid Passive Microfluidic Lab-on-Chip Device fabrication.

(iv) Device Development without any cleanroom facilities for resource limited environment.

(v) Possible effective techniques to compensate the dimensional errors for optimum device performance.

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